

NON-INVASIVE CHARACTERIZATION OF DISSOLVED OXYGEN DYNAMICS IN WATER-IN-OIL DROPLET MICROFLUIDICS – TOWARDS 3D MICRO TUMOR SPHEROIDS FOR HIGH THROUGHPUT CANCER DRUG SCREENING

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ABSTRACT

Oxygenation is a key parameter in tumor spheroid culturing. This work reports on the study of oxygen concentration and gas exchange dynamics in two-phase microfluidic droplet systems. Initial measurements of oxygen in single, oil-covered aqueous droplets in micro-wells show the feasibility of droplet-based sensing. We further introduce on-chip generation and trapping of individual droplets with controlled dissolved oxygen concentrations. Luminescence-based optical sensing is used to characterize the time-resolved dynamics of gas exchange between surrounding oil and micro-droplets where both phases exhibit pre-defined oxygen concentrations. This will help to determine suitable fluid combinations for oxygen-sensitive applications, such as high-throughput screening of drugs on micro-spheroids.

KEYWORDS: Microfluidic Droplets, Oxygen Measurement, Cancer Spheroids, RTDP,

INTRODUCTION

The desire to improve the efficacy of cancer drug screening has evoked the introduction of new cancer models which show in vivo-like responses to drug treatment and thus imitate the in vivo situation more closely [1]. This development is motivated by an increasing number of studies [2, 3] indicating that 3D cancer spheroids are more resistant to existing drugs when compared to the 2D cell cultures commonly used for drug screening. This difference in behavior observed between these models is partially attributed to the presence of hypoxic cells in the spheroid, absent in 2D models, and considered to contribute to resistance against radio- and conventional chemotherapy [4].

Droplet microfluidic devices have recently emerged as a promising high-throughput platform for the generation of cancer spheroids and the on-chip testing of therapeutic drugs [5]. By growing a 3D “micro tumor” in each droplet over several days of continuous culture a large number of spheroids and drug combinations can be tested simultaneously. One little explored additional parameter in this context is that of the gaseous microenvironment of the aqueous droplet in particular. The ability to control and measure the oxygen concentration of each droplet, and thus spheroid, will further extend the versatility of this platform [6].

Previously we have demonstrated the measurement and control of dissolved oxygen in laminar flow streams with the goal of providing a platform for the screening of hypoxia-targeted cancer drugs in 2D models [7]. Currently we are extending this system to the oxygen measurement and control in microfluidic droplet devices conducive to micro-spheroid formation. The study of oxygen exchange dynamics presented in this paper will enable the monitoring of the cellular metabolism during growth and the generation of droplet-based hypoxic oxygen environments for drug treatment.

EXPERIMENTAL

Single droplet gas exchange dynamics were initially characterized in 96 well plates by measuring the intensity change of a ruthenium-based fluorophore (tris (2,20-bipyridine) ruthenium (II) chloride hexahydrate (RTDP, Sigma)). Aqueous droplets of predetermined dissolved oxygen were pipetted into a well and covered with heavy mineral oil (#330760, Sigma). A Nikon Eclipse 80i fluorescence microscope with EX/EM 450(50) nm/590 nm filters and a digital camera (OrcaFlash4.0 V2, Hamamatsu) were used to record the intensity change of RTDP. Figure 1(a) shows a schematic of the measurement setup.

On-chip measurements were performed in a microfluidic chip containing a T-junction droplet generator and droplet-trapping bypasses replica-molded into polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning) using SU-8 photolithography. The device was plasma-bonded onto a glass microscope slide and interfaced with two syringe pumps providing oil and aqueous input streams, as shown in Fig. 1(b). Inside the device, individual droplets were rendered motionless by temporarily redirecting the main flow through bypasses indicated in Fig. 1(c, d), and their oxygen concentration was measured over extended periods. In both setups the initial concentration of each droplet was controlled by externally equilibrating the aqueous phase using either oxygen/nitrogen gas bubbling or sodium sulfite (Sigma) as an oxygen scavenger.

RESULTS AND DISCUSSION

An example of the oxygen dynamics of a water droplet surrounded by air-equilibrated mineral oil inside a 96 well plate is shown in Fig. 2. The oxygen concentration inside a droplet suspended in this type of oil was found to equilibrate over the duration of several minutes to hours depending on the initial concentration and method used to generate it. For

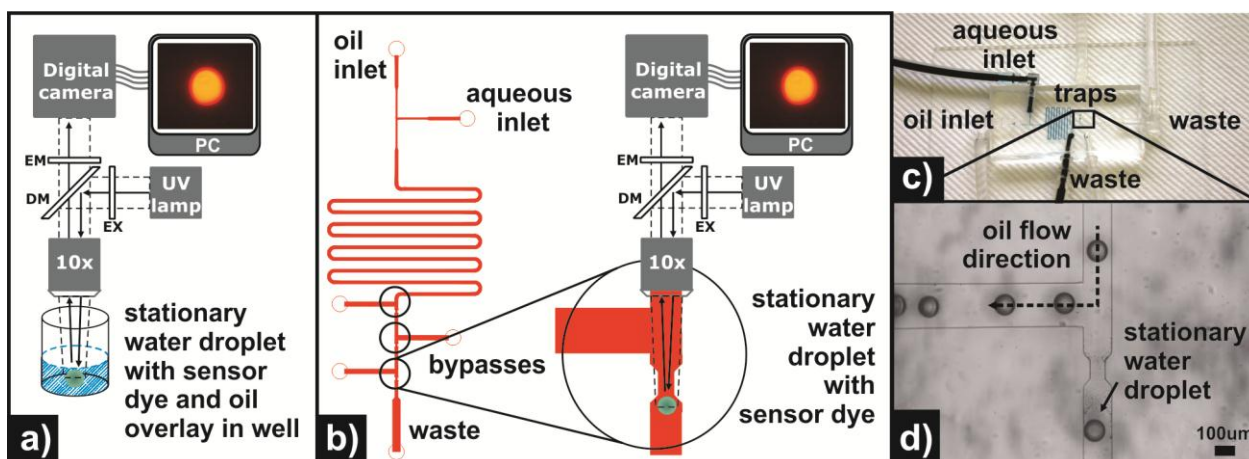


Figure 1: Schematics of the characterization and calibration setups: a) 1 μl water droplet in a 96 well plate with 100 μl mineral oil overlay. b) T-junction generated droplets trapped in bypasses. c) Optical micrographs of the PDMS device containing T-junction water-in-oil droplet generator and fluidic bypasses (traps). Blue color for visualization only. d) Close-up of mono-dispersed sample droplets filled with 1 mg/ml RTDP where single droplet is trapped for luminescent oxygen concentration measurement and oil flow directs droplets through bypass.

example, with the use of dissolved sodium sulfite as an oxygen scavenger hypoxic conditions inside the droplet could be maintained for up to 2 hours. However, a rapid return to air equilibrated conditions can be observed once all the sodium sulfite is converted to sodium sulfate. Starting conditions induced by bubbling oxygen or nitrogen gas through both phases on the other hand produce more gradual changes governed by diffusive processes.

A second parameter influencing the dynamic behavior is the type of oil used to encapsulate the water droplet. The use of different oils results in a change of oxygen solubility of the oil phase, which can range from a factor of 4 (light mineral oil) to a factor of 20 (perfluorinated oil) compared to the solubility of water. On contrary, the diffusion coefficient of oxygen in most oils used in droplet microfluidic systems is of the same magnitude as that of water [8]. Due to this the oxygen concentration in the water droplet quickly equilibrates towards the one of the oil. In droplet microfluidic devices this is further accelerated by the convection of the droplet formation process and the subsequent large surface-to-volume ratio of the droplet itself, as well as the volume of the oil compared to the volume of the droplet. Despite its importance for a wide variety of applications, oxygen exchange dynamics in the two phase system of a mono dispersed, spherical water droplets in a surrounding oil phase have so far only received limited attention [8, 9].

A similar dynamic was observed when aqueous droplets depleted with sodium sulfite and surrounded by air equilibrated mineral oil were measured on-chip (Fig. 3). The oxygen concentration inside a stationary $\sim 100\ \mu\text{m}$ diameter droplet was found to equilibrate within several minutes, while the passing droplets remained at initial hypoxic concentration. The much faster equilibration time is partially due to the reduction in diameter and thus surface-to-volume ratio, but also due to the surrounding oil being equilibrated with air instead of nitrogen. In the well plate experiments nitrogen bubbling was used to enable transitioning of the sample to the microscope. This crucial delay might also explain the difference between the initial intensity values of nitrogen equilibrated and sodium sulfite depleted droplets (Fig. 2). To better understand the influence of the various parameters we are currently in the process of extending our characterization procedure to less permeable chip materials and different carrier oils. Furthermore, previously developed thin-film oxygen sensors [7] will be integrated to provide reference sensors for aqueous and oil input and output streams.

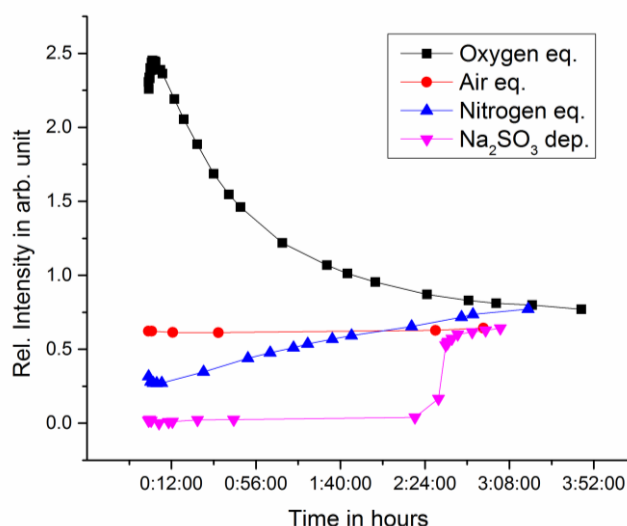


Figure 2: Oxygen exchange dynamics of a water-in-oil micro-droplet suspended in a 96 well plate. Graph shows the equilibration of the gas concentration inside the droplet with the device surrounding air as a function of time. Starting conditions correspond to oxygen bubbled water and oil (oxygen eq., \blacksquare), sodium sulfite induce hypoxia and nitrogen bubbled oil (Na_2SO_3 , \blacktriangledown), nitrogen bubbled water and oil (nitrogen, \blacktriangle) and air equilibrated (air, \bullet).

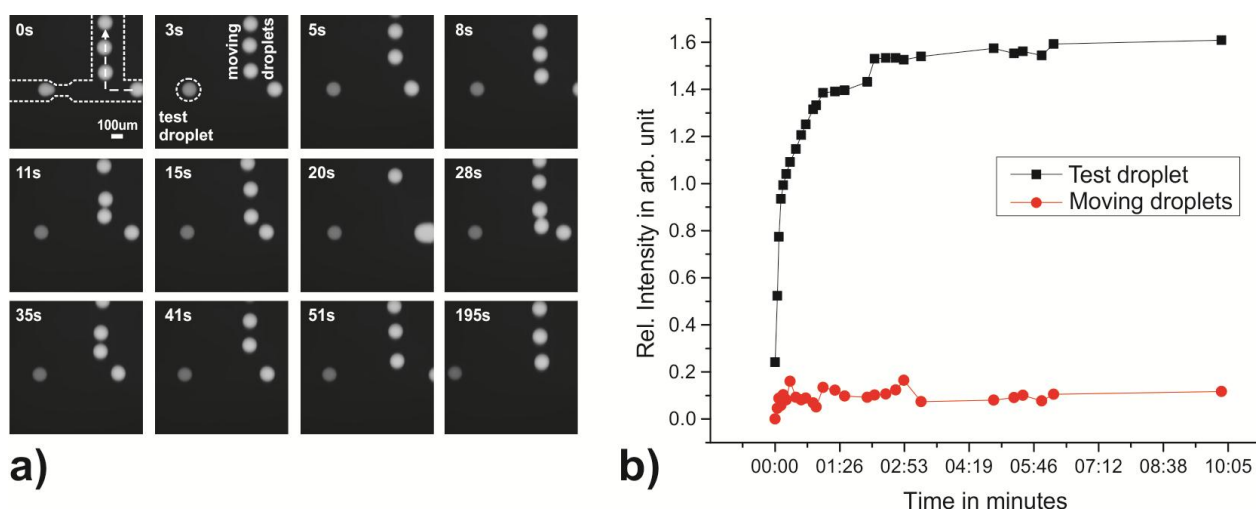


Figure 3: Oxygen exchange dynamics of a trapped water-in-oil droplet on-chip. *a)* Time sequence of fluorescence microscopy images showing a stationary droplet adjacent to droplets moving through a channel bypass. The mean intensity of the droplets corresponds to the dissolved oxygen concentration. *b)* Graph showing the equilibration of gas concentration inside the stationary droplet with the air equilibrated oil as a function of time. Starting conditions correspond to sodium sulfite induced hypoxia in water and air equilibrated oil.

CONCLUSION

We have shown the generation and trapping of 100 μm diameter aqueous micro-droplets and the characterization of oxygen exchange dynamics in relation to a carrier oil. Initial characterization and calibration of the measurement setup inside cell-culture micro-wells demonstrated the generation of stable hypoxic conditions inside an aqueous droplet over 2 h by use of sodium sulfite. Dynamic oxygen exchange to air-equilibrated water was visualized for different starting concentrations using fluorescence intensity-based measurement of oxygen concentration inside the droplets. A microfluidic device was fabricated to generate and trap individual droplets on chip. By rendering the droplet motionless by redirecting the main flow through a bypass structure, the oxygen dynamics of sodium sulfite depleted droplets were characterized. The current platform provides a useful tool to study the gas exchange dynamics of different oil-water combinations with respect to their suitability for use in spheroid-based drug screening devices.

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